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(54) Title: A METHOD FOR CORRECTING THE IMMUNE SYSTEM OF LIVE BODY

(57) Abstract: This invention belongs to a field of medicine and veterinary, namely, immunology, and relates to methods aimed at correcting the immune system of live body. The essence of the invention is in the immunocorrection of the body with the use of pharmacologically appropriate amino derivatives of 2,3-dihydrophthalazine-1,4-dione in their effective in their effective doses ranging from 0.2 μg to 1,000mg.

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A METHOD FOR CORRECTING THE IMMUNE SYSTEM OF LIVE BODY

This invention belongs to a new field of medicine and veterinary – immunology – and can be used for preventing and treatment of various diseases associated with immunopathologic changes, such as toxicoinfectious, oncologic, allergic and other diseases.

Immunostimulating and, conversely, immunodepressive effects are demonstrated by many well-known preparations. For example, tactivine, decaris and dibazole exert immunostimulating effects, while mercaptopurine and cyclophosphamide show the immunodepressive effects. However, the majority of well-known preparations exert unidirectional effects on the immune system.

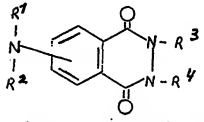
It is well . known, that sodium salt of 2-amino-1,2,3,4-tetrahydrophthalazine-1,4-dione, dihydrate, which is administered to patients with weak cellular immunity reactions (for example, patients with malignant tumors), causes activation of macrophages, interleukins and other acute phase proteins. This preparation is administered within a dose range of 10 to 1000 mg in the form of injections (for example, 100 mg in 1 ml of water) or per os – for example, 1000 mg in isotonic solution (RF Patent No. 2,113,222, Class A 61 K 31/04, 1998).

This invention presents a method of immunocorrection with application of a large group of pharmacologically adequate salts of amino-derivatives of 2,3-dihydrophthalazine-1,4-dione used in effective dose ranging from 0.2 μg to 1000 mg.

The difference of new invention from its prototype is in that it increases the number of immunocorrecting amino phthalazine dione compounds, and

considerably widens their field of application both as immunodepressants and immunostimulators in medicine and veterinary, with considerably increased range of effective doses as compared to the prototype.

This invention is using, as immunocorrecting agents, the well-known pharmacologically adequate compounds (which have been used previously for other purposes, for example, fungicides (US Patent No. 2,654,689, Class 514-248, published in 1953)) of the following general formula:



where R_1, R_2, R_3, and R_4 are H-, alkyl-, aryl-, alkylaryl-, atoms of metals or anions.

Such immunologically active compounds of this group include, for example:

- lithium, potassium, sodium, calcium, barium, magnesium and silver salts of 5-amino-2,3-dihydrophthalazine-1,4-dione;
- lithium, potassium, sodium, calcium, barium, magnesium and silver salts of 6-amino-2,3-dihydrophthalazine-1,4-dione;
- 5-amino-2,3-dihydrophthalazine-1,4-dione hydrochloride, sulfate, phosphate, citrate, tartrate, fumarate, oxalate, maleate, acetate, nitrate and hydrobromide;
- corresponding salts of 6-amino-2,3-dihydrophthalazine-1,4-dione;
- 5-methyl amino- and 6-methyl amino-2,3-dihydrophthalazine-1,4-diones and their corresponding, pharmacologically adequate, salts;
- 5,5-dimethyl amino-, 5,5-diethyl amino-, 5,5-dipropyl amino-, 5,5-dibutyl amino-, 5,5-dipentyl amino-, 6,6-dimethyl amino-, 6,6-diethyl amino-, 6,6-dipropyl amino-, 6,6-dibutyl amino- and 6,6-dipentyl amino-

2,3-dihydrophthalazine-1,4-diones and their corresponding, pharmacologically adequate, salts and
5-phenylamino- and 6-phenylamino-2,3-dihydrophthalazine-1,4-diones and their corresponding, pharmacologically adequate, salts.

The effective dose range of 0.2 μg to 1000 mg was determined experimentally. Selection of specific effective doses within this range for particular cases depends on the nature of disease, bodyweight and age of patient or animal; this is confirmed below by the data presented in examples and tables. Immunocorrectors may be administered in the form of injections, oral doses or external applications.

Immunogenic and allergenic properties of the advised compounds have been evaluated in accordance with Methodological Recommendations of Ministry of Health of the Russian Federation (R.M. Khaitov, I.S. Gushchin, B.V. Pinegin and A.I. Zebrev. "Experimentalnoye izucheniye immunotropnoy aktivnosti farmakologicheskikh preparatov" (Experimental study of pharmacologic preparations immunotropic activity), Vedomosti farmakologicheskogo komiteta (Pharmacological Committee Gazette), 1999, No. 1, pp. 31-36).

Mice belonging to various strains were used in this study depending on the applied method of investigation. All mice were supplied by "Stolbovaya" Russian Federation Academy of Medical Sciences (RF AMS) Brooder. Strains CBA and $C_{57}BL_6$ mice weighing 16 to 18 g were used in the studies of antibody production and antibody-producing cells.

Effects of the compounds on lymphoid cells proliferation and interleukin-2 induction *in vitro* were evaluated with the use of Strain BalB/c mice weighing 16 to 18 g. Animals of control and study groups were matched

by sex and weight. Each dose of preparation was tested on 10 animals; as a whole, more than 800 mice have been studied.

Allergenic effects of the preparations were evaluated on female guinea pigs weighing 250 to 300 g supplied by RF AMS Central Brooder of Laboratory Animals. A total of 70 guinea pigs have been studied. Intraperitoneal and intradermal injections of the preparations were used.

Production of antibodies and antibody-producing cells was studied on the model of ram erythrocytes placed in Olswer's solution. The erythrocytes were supplied by M.P. Chumakov Institute of Poliomyelitis and Viral Encephalites Brooder.

The invention can be illustrated by the following examples:

Example 1. Study of the effect of calcium salt of 5-amino-2,3-dihydrophthalazine-1,4-dione on the non-specific resistivity of the body.

Doses of the preparation ranging from 200 to 0.2 μg were injected intraperitoneally at ten-fold intervals in 0.5 ml of physiologic solution to Strain $C_{57}BL_6$ mice two hours before their infecting. Mice were infected with S. Typhi at a dose of $1 \cdot 10^4$ microbial cells; another group of animals was infected with Strain 264 E. coli at a dose of $1 \cdot 10^8$ microbial cells. Mice infected with the same microbial culture, but receiving no preparation, were used as controls. Lethal outcomes in mice were recorded every day for a period of 10 days.

It was revealed as a result of the experiment, that effects of the preparation on the non-specific resistivity of Strain $C_{57}BL_6$ mice depended on the administered dose and the used model of infection.

Doses of the preparation ranging from 200 to 2 μg caused no effects on the resistivity of mice infected with E. Coli, and their lifetime was the same as in controls (see Table 1).

Table 1.

Evaluation of the effects of calcium salt of 5-amino-2,3-dihydrophthalazine-1,4-dione on the non-specific resistivity of mice infected with E.Coli.

Dose of the preparation, μg	Number of mice	Number of lethal outcomes, for days							Mean lifetime
		1	2	3	4	5	6	7	
		Infecting with Strain 264 E.Coli at a dose of 10^8 microbial cells							
200	10	10	-	-	-	-	-	-	1.0
20	10	10	-	-	-	-	-	-	1.0
2	10	10	-	-	-	-	-	-	1.0
0.2	10	4	-	6	-	-	-	-	2.2
Controls	10	10							1.0

Thus, the dose of 0.2 μg ensured statistically significant (2.2-fold) increase in lifetime of experimental animals infected with E.Coli.

Example 2. Study of the effect of potassium salt of 5-amino-2,3-dihydrophthalazine-1,4-dione on phagocytosis *in vivo* and *in vitro*.

Strain $C_{57}BL_6$ mice received various doses of the preparation ranging from 200 to 2 μg, and 24 hours later they were tested. Intraperitoneal injection of 3 ml of 3 % peptone was made, and 2 hours later the animals were killed with chloroform. The mice were autopsied in aseptic conditions. Liquid was aspirated from abdominal cavity with a Pasteur's pipette and transferred to centrifuge test tubes; then, centrifuging was carried out for 10 minutes at 1,000 rpm. The sediment was re-suspended in Medium 199; number of cells was counted in Goryaev's chamber, and cell concentration was brought to $2 \cdot 10^6$ neutrophils/ml. Similar volume of Strain 1991 St. aureus was added to cells in 1:10 proportion and incubated at 37°C for 30 minutes. After

incubation, smears were prepared on slides, fixed in methanol for 20 minutes and processed with Romanovsky-Giemsa's stain for 30 minutes.

For evaluating the phagocytic activity of macrophages, the animals were tested on the third day after administration of peptone. Further testing was carried out similarly with that of neutrophils. The results were evaluated using a microscope at a magnification of 90x. Phagocytic index and phagocytic number were determined.

Results of the experiment have shown, that the preparation administered at a dose of 200 μg caused no stimulating effect on phagocytic cells, while doses of 2 μg and 20 μg ensured a statistically significant increase in the ability of phagocytic cells to intake microbial cells.

For evaluating the effect of the preparation on phagocytosis *in vitro*, blood sample was collected from donor's cubital vein in a test tube containing heparin at a dose of 10 units per 1 ml of blood. 0.8 ml of 3 % gelatin prepared based on Medium 199 were added to 2 ml of blood. The test tubes were incubated in a thermostat at 37°C for 20 minutes. Then, the supernatant containing cells was aspirated to a centrifuge tube. Cells were twice washed with Medium 199 with centrifuging at 1,000 rpm. 1 ml of Medium 199 was added to the sediment and neutrophils were counted in Goryaev's chamber. Then, cellular suspension containing $2 \cdot 10^6$ neutrophils in 1 ml was prepared based on Medium 199. Simultaneously, suspension of St. aureus in a concentration of $20 \cdot 10^6$/ml was prepared. After preliminary opsonization with an equal volume of pooled human plasma in a thermostat at 37°C for 20 minutes, the Staphylococcus suspension was centrifuged and washed with Medium 199. The preparation in 200, 20 and 2 μg/ml concentrations was added to mixture of equal volumes of neutrophils and staphylococci; Medium 199 was added to control test tubes. After incubation in a thermostat at 37°C for 30 minutes, the test tubes were centrifuged, and preparations were prepared in accordance with the procedure described above. Phagocytic index and

phagocytic number for study group were compared with the same for control group.

The obtained results are presented in Table 2:

Table 2.

Effects of various doses of potassium salt of 5-amino-2,3-dihydrophthalazine-1,4-dione on neutrophil phagocytosis *in vitro*.

Dose, μg	Study group		Control group	
	Phagocytic number	Phagocytic index	Phagocytic number	Phagocytic index
1	2	3	4	5
200	78	5.6	77	6.1
	85	4.7	86	5.1
	84	9.1	85	8.7
	71	6.4	72	7.2
	67	7.4	68	8.1
Mean	77±0.26	6.0±0.04	77.6±0	7.0±0.04
20	80	3.9	76	4.2
	96	2.8	66	3.5
	88	4.6	82	6.6
	94	6.8	82	5.6
	84	13.2	83	5.0
	96	9.1	92	10.0
	86	16.3	74	5.5
	84	5.2	62	4.4
Mean	89±1.25	7.8±0.04	77±0.35	5.6±0.1
	88	6.4	87	6.2
	92	4.5	80	7.3
	78	7.4	30	5.8
	84	6.4	80	5.5
	92	5.6	80	9.1
Mean	86±0.32	6.0±0.16	71.4±0	6.8±0.2

The obtained data show, that the injected doses of the preparation ranging from 200 to 2 μg caused stimulating effects on phagocytic activity of macrophages in mice, while the dose of 200 μg caused no effect on phagocytic activity *in vivo*.

It was revealed in the *in vitro* experiments, that the studied preparation used at the doses of 20 and 2 μg caused statistically significant increase in the ability of neutrophils population to intake microbial cells and demonstrated practically no effect on their digestive activity.

Example 3. Study of the effect of sodium salt of 5-amino-2,3-dihydrophthalazine-1,4-dione on the humoral immune response.

Mice were immunized intraperitoneally with ram erythrocytes washed with physiologic solution; the erythrocytes were injected at a dose of $5 \cdot 10^6$ cells. Other groups of animals received ram erythrocytes and doses of the preparation ranging from 200 to 0.2 μg at ten-fold intervals. When productive phase of humoral immunity response was studied, the preparation was administered to mice on the fifth day after their immunization with erythrocytes. Blood samples of the mice were collected on the 7th, 14th and 21st day after immunization. Antibodies were determined using a reaction of hemagglutination. Two-fold dilutions of mice blood sera were studied in 96-well plates for immunological reactions with U–shaped bottoms in 25-μl volumes. 25 μl of physiologic solution were added to control well. 25 μl of 1 % solution of ram erythrocytes were added to each well. The plates were incubated in a thermostat at 37°C for 2 hours. The last dilution of the studied serum demonstrating positive result was considered as a titer. The control well had to be negative.

When the effect of the preparation on inductive phase of immune response was studied, the preparation was administered to mice simultaneously with ram erythrocytes. Blood was collected from mice on the 7th, 14th and 21st

day after immunization. Antibodies were determined using a reaction of hemagglutination as described above.

Results obtained after simultaneous administration of ram erythrocytes (RE) and the preparation are shown in Tables 3 and 4. Strain $C_{57}BL_6$ animals of low reaction showed a tendency to increase in titers of hemagglutinins on the 21st day after administration of 0.2 or 20 μg of the preparation to each mouse. Study of the effect of the preparation on productive phase of immune response of mice revealed a suppressive effect of all doses of the preparation on Strain CBA mice of high reaction, as well as a tendency to increase in the level of hemagglutinins on the 21st day after administration of the preparation to low-reaction Strain $C_{57}BL_6$ mice:

Table 3.

Titers of hemagglutinins after simultaneous administration of sodium salt of 5-amino-2,3-dihydrophthalazine-1,4-dione and RE.

Dose of the preparation, μg	Strain CBA mice			Strain $C_{57}BL_6$ mice		
	7 days	14 days	21 days	7 days	14 days	21 days
0.2	112 ± 106	168 ± 109	15 ± 13	59 ± 58	50 ± 48	266 ± 200
2	28 ± 26	154 ± 97	10 ± 8	154 ± 57	256 ± 0	24 ± 20
20	96 ± 37	256 ± 0	83 ± 70	91 ± 64	512 ± 0	560 ± 160
200	35 ± 33	75 ± 59	46 ± 32	512 ± 0	44 ± 42	160 ± 138
RE	112 ± 106	512 ± 0	24 ± 12	154 ± 57	256 ± 0	27 ± 15
Controls	0	4 ± 0	0	0	4 ± 0	0

Table 4.

Titers of hemagglutinins revealed in mice receiving the preparation five days after immunization with RE.

Dose of the preparation, μg	Strain CBA mice			Strain $C_{57}BL_6$ mice		
	7 days	14 days	21 days	7 days	14 days	21 days
0.2	0	9 ± 7	0	0	2 ± 0	35 ± 10
2	10 ± 8	173 ± 110	22 ± 10	13 ± 11	54 ± 50	226 ± 201
20	0	256 ± 0	23 ± 10	26 ± 19	256 ± 0	304 ± 105
200	28 ± 13	158 ± 120	47 ± 30	0	96 ± 42	120 ± 95
RE	111 ± 106	512 ± 0	24 ± 12	154 ± 57	256 ± 0	27 ± 15
Controls	0	4 ± 0	0	0	4 ± 0	0

Thus, the ability of sodium salt of 5-amino-2,3-dihydrophthalazine-1,4-dione to change antibody production depending on its dose and initial immunologic reactivity of the body has been revealed. Administration of the preparation during productive phase, that is, on the 5th day after injection of antigens (ram erythrocytes), resulted in inhibition, with wide range of doses, the production of antibodies on the 7th and 14th days after immunization of Strain CBA mice, which are, genetically, highly sensitive to ram erythrocytes. In low-sensitive Strain $C_{57}BL_6$ mice, The preparation caused significant increase in the titers of hemagglutinins in low-sensitive Strain $C_{57}BL_6$ mice on the 21st day of the experiment.

Example 4. Study of the effect of sodium salt of 6-amino-2,3-dihydrophthalazine-1,4-dione on the cellular immune response.

To evaluate the effect of the preparation on cellular immunity reaction, delayed hypersensitivity reaction (DHR) was used. The mice were sensitized by subcutaneous injection of $1 \cdot 10^7$ ram erythrocytes contained in a volume of

20 µl. Doses of the preparation ranging from 0.2 to 2,000 µg were injected at ten-fold intervals simultaneously with sensitizing and shocking dose of the antigen. Shocking dose of ram erythrocytes ($1 \cdot 10^8$) was injected under aponeurotic plate of left hind paw on the 5th day after sensitization. Injection of 20 µl of physiologic solution in the right paw served as control. Intensity of inflammatory reaction was recorded 24 hours after administration of shocking dose of the antigen. For this purpose, the mice were killed and, immediately after this, both paws were amputated at the level of ankle joint and weighed on torsion balance. Reaction index (RI) was determined as the difference of masses of study (S) and control (C) paws using the following formula:

$$RI = \frac{S - C}{C} \times 100\ \%$$

This study evaluating the effects of the preparation on cellular immune response based on DHR has revealed a tendency to increase in reaction index for mice of both strains as the dose of the preparation decreased (see Table 5). It should be noted, that the increase in reaction index accompanying the decrease in the dose of preparation was especially prominent in low-reaction Strain $C_{57}BL_6$ mice. Administration of the preparation at a dose of 200 µg to high-reaction Strain CBA mice resulted in inhibition of DHR.

Table 5.

Effect of sodium salt of 6-amino-2,3-dihydrophthalazine-1,4-dione on delayed hypersensitivity reaction (DHR) in mice

Dose of the preparation, µg	Reaction index	
	Strain CBA mice	Strain $C_{57}BL_6$ mice
2	11 ± 8	19 ± 9
20	10 ± 5	16 ± 9
200	2 ± 1.5	15 ± 6
Control	6 ± 3.5	12 ± 7

Thus, administration of 200 μg of the preparation to Strain CBA mice suppressed the development of DHR. All other doses of the preparation (20 to 2 μg) showed no effect on the development of DHR in mice of both oppositely reacting strains.

Example 5. Study of the effect of 5-amino-2,3-dihydrophthalazine-1,4-dione hydrochloride on lymphoid cells proliferation.

Spleens of the animals were used for carrying out the immunologic reactions. The organs were cut and homogenized in a glass homogenizer. The homogenate was filtered through stainless steel filters with pores measuring 50 to 100 μm in diameter and then washed three times in a medium designed for centrifuging (CM) consisting of Medium 199 with 5 % embryonic calf serum (produced by RF AMS N.F. Gamaleya NIIEM), 1 mM of HEPES buffer solution and 50 μg/ml gentamicin. The suspensions were centrifuged in a K23 centrifuge at 1,500 rpm at 4 °C for 10 minutes.

The suspensions were diluted 100-fold with 3 % acetic acid contrasted with methylene blue, and cells were counted in Goryaev's chamber. Cell viability was evaluated with aid of 0.1 % trypan blue in physiologic solution.

Results of the study of mitogenic effects of the preparation, as well as the effects of the preparation on proliferation caused by T-cell (KonA) and B-cell (LPS) mitogens, are presented in Table 6.

Table 6.

Effect of 5-amino-2,3-dihydrophthalazine-1,4-dione hydrochloride on lymphoid cells proliferation.

Concentration of the preparation	Mitogen added to spleen cell culture		
	-	ConA	LPS
-	3534 ± 563	17896 ± 2080	18874 ± 355
50 μg/ml	2576 ± 237	11860 ± 1566	13232 ± 928
500 μg/ml	1763 ± 94	1323 ± 192	3870 ± 308
2.5 mg/ml	249 ± 93	195 ± 27	251 ± 44
12.5 mg/ml	369 ± 56	178 ± 28	256 ± 40

As the presented data show, the preparation has no mitogenic properties within the studied dose range (50 μg/ml to 12.5 mg/ml). However, this preparation, when used in higher concentrations, inhibited both the spontaneous proliferation of spleen cells and the proliferation induced by non-specific mitogens (ConA and LPS). LPS = Lipopolysaccharide ConA = Concanavaline A

Example 6. Study of the effect of sodium salt of 5-methylamino-2,3-dihydrophthalazine-1,4-dione on functional activity of natural killers.

Labeled cells of mice of myeloblastoid Strain K-562, as well as mononuclear cells of 10 healthy blood donors and 10 patients with various diseases with low and high levels of cytotoxicity, were used for the study of effects of various concentrations of the preparation on cytotoxic activity of natural killers ($CD3^-$, $CD16^+$ and $CD56^+$).

The used Strain K-562 cells to effector cells ratio was 1:25 (100 μl of labeled cells and 100 μl of mononuclear cells). The studies were carried out using 96-well plates; incubation for 16 to 24 hours in a CO_2-incubator at 37 °C was carried out. Then, the contents of the wells was transferred to filters, washed, dried and placed in solution with scintillation liquid with consecutive

determination of index using a β-counter. Cytotoxic index (CI) was calculated using the following formula:

$$CI = 1 - \frac{(A-B)}{(C-B)} \times 100,$$

where A = target cells radioactivity in the presence of effector cells;

B = radioactivity after cells treatment with tribon X-100 (maximum value);

C = radioactivity of target cells in the absence of effector cells.

The obtained results are shown in Tables 7 and 8 below.

Table 7.

Effects of various concentrations of sodium salt of 5-methylamino-2,3-dihydrophthalazine-1,4-dione on cytotoxic index depending on control level of CI.

Concentration of the preparation, μg/ml	CI, %
Control	41.0 ± 3.0
2.5	41.9 ± 2.8
5.0	38.8 ± 3.1
10.0	40.8 ± 3.0
25.0	41.4 ± 3.0
50.0	39.1 ± 4.0
75.0	37.7 ± 4.1
150.0	41.0 ± 3.5
300.0	39.0 ± 3.1

The obtained results have shown, that various concentrations of the preparation (within 2.5 to 300 μg/ml range) did not cause significant modification of natural killers cytotoxicity when normal level of CI was present.

Table 8.

Effects of low concentrations of sodium salt of 5-methylamino-2,3-dihydrophthalazine-1,4-dione on cytotoxic index depending on control level of CI.

Concentration of the preparation, μg/ml	Cytotoxic index level, % (M)		
	Medium	High	Low
Control	41.0 ± 0.8	64 ± 0.8	20.7 ± 1.0
2.5	41.9 ± 0.9	57 ± 0.7	34 ± 1.3
5.0	38.8 ± 0.34	54 ± 0.8	30 ± 1.2
Number of observations	10	10	10

It follows from the data presented in Table 8, that the preparation, when used in 2.5 and 5.0 μg/ml doses in conditions of high initial level of CI, caused statistically significant inhibition of cytotoxicity, while in conditions of low initial level of CI it caused statistically significant stimulation of functional activity of natural killers, that is, it demonstrated the ability to modulate the cytotoxicity depending on its initial level.

Example 7. Study of anaphylactogenic activity of sodium salt of 5-amino-2,3-dihydrophthalazine-1,4-dione.

The preparation under study was injected to three groups of guinea pigs in accordance with the following schedule: first 0.1-ml injection of various doses (1 μg, 10 μg and 100 μg) was made subcutaneously; second 0.1-ml injection of the preparation was made on the next day intramuscularly in the thigh; and the third 0.1-ml injection was made one day later. On day 21, the shocking injection of main pharmacological effect with single application was made. The obtained results are shown in Table 9.

Table 9.

Expression of anaphylactic shock

Sensitization with the preparation, μg	Shocking injection, μg	Number of animals	Expression of anaphylactic shock					Weigl's index
			-	+	++	+++	++++	
100	100	10	5	1	1	-	-	0.3
10	100	10	7	2	-	-	-	0.2
1.0	100	10	10	-	-	-	-	

Thus, the expression of anaphylactic activity of the studied preparation was very weak; it was demonstrated only for high doses of the preparation.

Apart from the studies on animals described above, clinical studies of immunocorrection activity of amino derivatives of 2,3-dihydrophthalazine-1,4-dione represented by sodium salt of 5-amino-2,3-dihydrophthalazine-1,4-dione in the treatment of various diseases in humans, in particular, anti-inflammatory immunocorrecting activity in the treatment of ulcers, were carried out.

Examples 8 and 9 illustrate the results of clinical studies.

Example 8. Patient K aged 35 years had a chronic recurrent ulcer on the antero-inferior wall of duodenal bulb measuring about 1.5 cm in diameter and about 0.5 cm in depth; another ulcer measuring 0.3 cm in diameter was present on the opposite wall ("kissing ulcer"). Concomitant catarrhal bulbitis was present. The patient partly lost her working capacity due to this disease.

Treatment: Periulceral injection of 100 mg of sodium salt of 5-amino-2,3-dihydrophthalazine-1,4-dione was made. One more injection of the preparation was made on the 7th day. The patient also received the preparation in Almagel suspension and, since the 7th day, in Maalox (in view of constipations). She was simultaneously treated with omeprazol in accordance with usual regimen and, since the 7th day, De-nol with trichopol.

Significant weakening of pain was noted on the 2nd day of treatment; the pain completely disappeared on the 7th day. By the 7th day, decrease in ulcer dimensions by 1/3, clearing of the bottom of ulcerous defect, epithelialization of the upper wall of ulcer and decrease in the manifestations of bulbitis were noted. By the 15th day of treatment, the dimensions of ulcerous defect decreased by 2/3. The ulcer completely scarred by the 21st day. The patient received a total of 1,000 mg of the preparation in the course of treatment.

Example 9. Study of the efficacy of potassium salt of 5-amino-2,3-dihydrophthalazine-1,4-dione in the treatment of acute intestinal infections (AII).

Efficacy of the preparation in decreasing the gravity of manifestations of various forms of AII was evaluated in accordance with the used program of study with aid of a three-point scale. In 19 of 20 patients, the intoxication syndrome resolved within 1 to 3 days of the disease ("complete decrease" in the gravity of disease), and one patient demonstrated "insignificant decrease" in the course of the first days and "complete decrease" by the 5th day of treatment.

After the study was finished, general evaluation of clinical efficacy and safety of the preparation based on a 4-point scale, depending on nosologic forms, was carried out. Results of this evaluation are presented in Table 10.

Table 10.

Efficacy of potassium salt of 5-amino-2,3-dihydrophthalazine-1,4-dione in the treatment of various forms of AII.

Form of disease	Total number of patients	Efficacy of the preparation			
		Excellent	Good	Satisfactory	No effect
Acute dysentery	9	1	7	1	-
Salmonellosis	3	1	2	-	-
Alimentary toxoinfection	4	1	3	-	-
Acute gastroenteritis	4	2	1	1	-
Total:	20	5	13	2	-

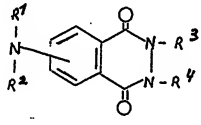
Thus, the preparation has demonstrated high efficacy in the treatment of all 20 patients with various forms of AII; the preparation showed "excellent" or "good" effect in 90 % of patients (18 patients).

It follows from the data presented above, that the studies of immunotropic activity of amino derivatives of 2,3-dihydrophthalazine-1,4-dione have revealed their dose-dependent immunocorrecting activity within the 0.2 μg to 1,000 mg dose range.

Claims:

1. Pharmacological preparation for correcting the immune system of live bodies comprising as immunocorrecting agent at least an amino derivative of 2,3-dihydrophthalazine-1,4-dione or a pharmacologically adequate salt thereof.

2. Pharmacological preparation according to claim 1, comprising as immunocorrecting agent at least a compound according to the following general formula:



where, R_1, R_2, R_3 and R_4 are H-, alkyl-, aryl-, alkylaryl-, atoms of metals or anions, and/or a derivative and/or a salt thereof.

3. Pharmacological preparation according to one of the claims 1 or 2, wherein the immunocorrecting agent is a lithium, potassium, sodium, calcium, barium, magnesium and/or a silver salt of 5-amino-2,3-dihydrophthalazine-1,4-dione.

4. Pharmacological preparation according to one of the claims 1 or 2, wherein the immunocorrecting agent is a lithium, potassium, sodium, calcium, barium, magnesium and/or a silver salt of 6-amino-2,3-dihydrophthalazine-1,4-dione.

5. Pharmacological preparation according to one of the claims 1 or 2, wherein the immunocorrecting agent is a 5-amino-2,3-dihydrophthalazine-1,4-dione hydrochloride, -sulfate, -phosphate, -citrate, -tartrate, -fumarate, -oxalate, -maleate, -acetate, -nitrate and/or -hydrobromide.

6. Pharmacological preparation according to one of the claims 1 or 2, wherein the immunocorrecting agent corresponds to a salt of 6-amino-2,3-dihydrophthalazine-1,4-dione.

7. Pharmacological preparation according to one of the claims 1 or 2, wherein the immunocorrecting agent corresponds to 5-methyl amino- and/or 6-methyl amino-2,3-dihydrophthalazine-1,4-dione and/or corresponding, pharmacologically adequate salts.

8. Pharmacological preparation according to one of the claims 1 or 2, wherein the immunocorrecting agent is 5,5-dimethyl amino-, 5,5-diethyl amino-, 5,5-dipropyl amino-, 5,5-dibutyl amino-, 5,5,-dipentyl amino-, 6,6-dimethyl amino-, 6,6-diethyl amino-, 6,6-dipropyl amino-, 6,6-dibutyl amino- and/or 6,6-dipentyl amino-2,3-dihydrophthalazine-1,4-dione and/or corresponding, pharmacologically adequate salts.

9. Pharmacological preparation according to one of the claims 1 or 2, wherein the immunocorrecting agent is 5-phenylamino- and/or 6-phenylamino-2,3-dihydrophthalazine-1,4-dione and/or corresponding, pharmacologically adequate salts.

10. Use of the pharmacological preparation according to one of the claims 1 to 9 for correcting the immune system

of live bodies with the aid of an immunocorrecting agent as defined in one of the claims 1 to 9, which is distinguished by the use as immunocorrectors of pharmacologically adequate amino derivatives of 2,3-dihydrophthalazine-1,4-dione and/or a salt thereof in effective dose ranging from 0.2 µg to 1000 mg.

11. Use of the pharmacological preparation according to one of the claims 1 to 9, using as immunocorrectors pharmacologically adequate metallic salts of amino derivatives of 2-3-dihydrophthalazine-1,4-dione.

12. Pharmacological preparation according to one of the claims 10 or 11, using as immunocorrecting agent a derivative as claimed in claim 3.

13. A method for correcting the immune system of live body with the aid of amino dihydrophthalazine compounds, which is distinguished by the use, as immunocorrectors, of pharmacologically adequate amino derivatives of 2,3-dihydrophthalazine-1,4-dione in effective doses ranging from 0.2 µg to 1000 mg.

14. A method, in accordance with Example 1, which is distinguished by preferential use, as immunocorrectors, of pharmacologically adequate metallic salts of amino derivatives of 2,3-dihydrophthalazine-1,4-dione.